Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Assessment of benefits and risks of probiotics in processed cereal-based baby foods

Lactobacillus paracasei ssp. *paracasei* F19

Opinion of the Panel on Nutrition, Dietetic products, Novel food and Allergy and Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety

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Summary

The Norwegian Scientific Committee for Food Safety (VKM) has appointed an *ad hoc*-group of experts to answer a request from the Norwegian Food Safety Authority regarding benefit and risk assessment of *Lactobacillus paracasei* ssp. *paracasei* F19 (F19) in processed cereal-based baby foods intended for small children 1-3 years. This assessment is based on the literature provided by the notifier as well as that found by a MEDLINE search.

A notification regarding two products of processed cereal-based baby foods (hereafter called cereals), intended for small children and supplemented with the bacterium F19 initiated this work.

A daily supply of a monoculture of a particular bacterial strain in large quantities to an age group without a fully established intestinal flora, may have unknown adverse effects. There are however, to our knowledge, no studies investigating possible short or long term adverse health effects of F19 in processed cereal-based baby food given to children 13 months onwards.

The documentation and information provided by the notifier regarding the genetic stability of F19 in the two products during processing and storage, is considered insufficient and does not allow any conclusions to be drawn.

Moreover, the documentation obtained is not conclusive regarding the antibiotic resistance pattern of the bacterial strain used in the products in question, as the information on different antibiotics is partly inconsistent. The information about specific localization (chromosomal, plasmid) of the resistance genes is not sufficient.

Studies demonstrate that F19, as well as other bacterial strains considered probiotic, is able to "crosstalk" with enterocytes in mice and that the result of the "crosstalk" depends upon the microbiota present. Whether F19 has a similar "crosstalk-profile" in humans is unknown. However, as the strain is originally of human origin, it seems reasonable to assume that such "crosstalk" may occur. Thus, before giving F19 daily for months and years, it seems reasonable to ask for additional molecular and physiological studies to unravel the functional impact of possible changes in genetic expression in children.

Lactobacillus infections do occasionally occur, mainly as bacteremia, endocarditis and localized infections (e.g. abscesses, peritonitis, and meningitis) in patients with severe underlying diseases. Most of them are elderly, but children are not excluded. The species most often isolated are *L. casei* and L. *rhamnosus*, followed by *L. paracasei*.

The increasing use of immunosuppressive therapy and broad spectrum antibiotics which are ineffective against *Lactobacillus,* might increase the importance of these bacteria as possible pathogens. In order to be able to draw any conclusions regarding beneficial effects of F19, there is a need for randomized placebo-controlled studies in larger populations and in the relevant age group.

According to EFSA, *Lactobacillus paracasei* ssp. *paracasei* F19 is sufficiently characterized. The documentation provided is, however, not sufficient to claim positive health effects and thus F19 is not proven to be probiotic.

There are no published dose-response studies of F19 in children, neither regarding survival of F19 in the gastrointestinal tract, nor possible negative health effects. Thus the potential for negative health effects as e.g. spreading of antimicrobial resistance or unfavourable impact on the genetic expression in children related to the frequency and/or dose of a monoculture of F19 cannot be assessed.

Norsk sammendrag

Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag fra Mattilsynet utarbeidet en nytte- og risikovurdering av *Lactobacillus paracasei* ssp. *paracasei* F19 (F19) tilsatt i barnegrøt til barn i alderen 1-3 år. For å besvare oppdraget nedsatte VKM en *ad hoc*-gruppe. Vurderingen er basert på gjennomgang av litteratur tilsendt fra virksomheten og MEDLINE litteratursøk.

Bakgrunnen for oppdraget er en innmelding av to barnegrøter tilsatt F19 i Norge.

Daglig tilgang på en monokultur av en spesiell bakteriestamme i store mengder til en aldersgruppe uten fullt etablert tarmflora kan gi uønskede effekter. Så langt er det ikke gjort studier som undersøker kort – eller langtidsbivirkninger av F19 brukt i barnegrøt til barn eldre enn 13 måneder.

Dokumentasjonen oversendt fra søker angående genetisk stabilitet av F19 i de to produktene anses som utilstrekkelig slik at ingen konklusjoner kan trekkes.

Videre ansees ikke dokumentasjonen vedrørende antibiotika resistensmønsteret for bakteriestammen som er brukt i produktet klar siden informasjonen for enkelte antibiotika varierer. Informasjon om lokalisering av resistensgener (kromosomalt, plasmid) er heller ikke tilstrekkelig.

Studier viser at F19 og andre probiotiske stammer kan ”kommunisere” med enterocyter i mus og resultatet av ”samtalen” avhenger av tilstedeværelsen av mikrobiota. Om F19 har samme ”samtaleprofil” hos mennesker er uvisst. Stammen er imidlertid av human opprinnelse, så slik ”kommunikasjon” kan være sannsynlig. Før F19 tilføres daglig i måneder og år, er det rimelig å be om molekylære og fysiologiske studier, for å avsløre den funksjonelle virkningen av mulige endringer i barns genetisk uttrykk. *Lactobacillus*-infeksjoner forekommer en sjelden gang som bacteriemi, endokarditt og lokale infeksjoner (for eksempel absesser, peritonitt, meningitt) hos pasienter med alvorlige underliggende sykdommer. Mange av dem er eldre, men barn er ikke unntatt. Stammene som oftest blir isolert er *L. casei* og L. *rhamnosus*, deretter *L. paracasei*.

Den økende bruken av immunosuppressiv behandling og bredspektret antibiotikum som ikke virker mot *Lactobacillus* kan øke betydningen av disse bakteriene som mulig sykdomsfremkallende. For å kunne trekke konklusjoner om nytte-effekter av F19 er det nødvendig å gjøre randomiserte placebokontrollerte studier i større populasjoner og i relevante aldersgrupper.

Ifølge EFSA er *Lactobacillus paracasei* ssp. *paracasei* F19 tilstrekkelig karakterisert. Dokumentasjonen som finnes er allikevel ikke god nok til å hevde positive helseeffekter, og det er derfor ikke bevist at F19 har probiotisk effekt.

Det finnes ingen publiserte dose-respons studier av F19 hos barn, verken når det gjelder overlevelse av F19 i mage-tarmkanalen eller mulige negative helseeffekter. Således kan potensialet for negative helseeffekter, som for eksempel spredning av antimikrobiell resistens eller uønsket påvirkning på det genetiske uttrykk hos barn relatert til frekvens og/eller dose av monokultur av F19, ikke vurderes.

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Background

In 2008 and 2009, the Norwegian Food Safety Authority (Mattilsynet) received a notification regarding two types of processed cereal-based baby foods containing the probiotic microorganism *Lactobacillus paracasei* ssp. *paracasei* F19.

The addition of probiotics of different bacterial species and strains to regular foods, including infant formulas and baby foods, is increasing.

Foods with added probiotics, and in particular milk products for children, are being increasingly promoted by the food industry. It has been claimed that these microorganism can play an important role in immunological, digestive and respiratory functions and could have a significant effect in alleviating infectious disease in children as well as adults.

The notifier has provided documentation on the origin of the bacterial strain *Lactobacillus paracasei* ssp. *paracasei* F19, and the manufacturing process. Documentation on microbial and chemical safety was included in the notification.

In May 2009, the Norwegian Food Safety Authority requested the Norwegian Scientific Committee for Food Safety (VKM) to make an assessment of the benefits and risks of the addition of *Lactobacillus paracasei* ssp. *paracasei* F19 in two processed cereal-based baby foods, and an *ad hoc*-group of experts was appointed of the Panel on biological hazards and the Panel on nutrition, dietetic products, novel food and allergy with the mandate to draft this assessment.

VKM has previously published four assessments of the use of probiotics; Assessment of benefits and risk of probiotics in processed cereal-based baby foods - *Bifidobacterium lactis* Bb12 (VKM, 2010), Benefit and risk assessment of the use of probiotics for patients in hospitals (VKM, 2009), Risk assessment on use of *Lactobacillus rhamnosus* (LGG) as an ingredient in infant formula and baby foods II (VKM, 2007) and Risk assessment on use of *Lactobacillus rhamnosus* (LGG) as an ingredient in infant formula and baby foods (VKM, 2005).

Furthermore, the European Food Safety Authority (EFSA) has assessed and dismissed several health claims and nutrition claims related to probiotics.

All these reports and opinions have been valuable background documents in this assessment.

Terms of reference

Translated from the Norwegian terms of reference[1]:

1. What benefit can children (1 to 3 years) have from consuming processed cereal-based food containing *Lactobacillus paracasei* ssp. *paracasei* F19?
2. What impact can the addition of *Lactobacillus paracasei* ssp. *paracasei* F19 to processed cereal-based food for children (1 to 3 years) have on the development of allergy?

[1] Norwegian terms of reference are listed in Appendix I

3. Are there any contraindications regarding the consumption of processed cereal-based food containing *Lactobacillus paracasei* ssp. *paracasei* F19 for children (1 to 3 years)?
4. Is there any risk that the consumption of processed cereal-based food containing *Lactobacillus paracasei* ssp. *paracasei* F19 can lead to a spreading of antimicrobial resistance to other members of the gut microbiota?
5. What possible negative health effects are correlated to the daily consumption of processed cereal-based food containing *Lactobacillus paracasei* ssp. *paracasei* F19? Furthermore, will an increased consumption (amount and frequency) result in pronounced effects?
6. Do the products contain ingredients that can have prebiotic effect?

1 Information provided by the notifier

Two processed cereal-based products (oat cereal and multi-grain cereal) with F19 have been notified.

The literature provided by the notifier regarding the origin of *Lactobacillus paracasei* ssp. *paracasei* F19 (F19) is not consistent, see section 3.1.

The bacterial culture is supplied by Christian Hansen (Denmark). The strain has been deposited at Belgian Coordinated Collections of Microorganisms - BCCM, LMG collection, with the accession No. LMG P-17806.

The number of viable cells of the culture in a portion of the cereal powder has been given as 10^8 cfu according to information from the notifier.

1.1 Food/constituent as stated by notifier

The oat cereal contains 34% full corn cereal of which 22.7% is oat flour. The multigrain cereal contains 40% full corn cereal, of which 10.5% is oat flour and 9.5% is sifted wheat flour. Both products contain dairy based powders and fruit additions. Vitamins and minerals are added. The product contains the probiotic bacterium F19.

Table 1: Nutritional content of the two notified cereal products

Nutritional content /100g	Multi-grain cereal with apple & pear + probiotics	Oat-cereal with probiotics
Energy kJ	1800	1850
Protein	15 %	15 %
Carbohydrate	55 %	54 %
- of which, sugars	*23 %*	*29 %*
Fat	17 %	18 %
- saturated	*5 %*	*5.50 %*
- mono-unsaturated	*7 %*	*7.50 %*
- polyunsaturated	*5 %*	*4.50 %*

The cereal powder is to be prepared by stirring the cereal mixture into hot water (approximately 50°C). The consumer is advised to avoid too hot water as the "The bacteria culture does not tolerate heating above 60°C". The Norwegian labelling text is given in Appendix II.

1.2 Wording of the health claims as proposed by the notifier2

Foods containing probiotics are commonly marketed with different nutritional and health claims. Health claims related to probiotics are currently and continuously assessed by EFSA. So far none of the health claims related to probiotics have been accepted as sufficiently documented by EFSA, (see list of EFSA opinions in chapter 2 Data sources). The claimed health effects of the two products in question are similar to those assessed by EFSA for other probiotic bacteria.

The following labelling claims are suggested by the notifier for multi-grain cereal and oat cereal:

[2] Free translation of Norwegian labelling text and text from notifiers website. Norwegian labelling and text from notifiers website is given in Appendix II

- "Contains probiotic lactic acid bacteria that have scientifically documented properties."
- "Probiotic lactic acid bacteria are naturally found in our intestines and an extra addition of these can contribute to maintain a healthier and more robust gut flora. The digestive tract is the body's most important immune organ (*lit*. system)."
- "Probiotics keep the gut in balance!"
- "The cereal is appropriate for children from 1 ½ years, and with the addition of probiotic bacteria it is well-suited for children right up to school age" (on the oat cereal only).

Additional information about probiotics in the notified processed cereal-based food is given on the notifiers website:

- "Will improve the immune system."
- "Probiotics have been shown to have a healthy effect on children and adults."
- "A complex intestinal flora provides protection against colonisation by harmful bacteria, by creating competition for nourishment and space. It is these abilities of intestinal bacteria to stimulate the immune system and to compete with harmful bacteria that have led to the development of probiotics."
- "Probiotic lactic acid bacteria are harmless bacteria that may be added to food and drink. Research has shown that some probiotics have a good effect on certain infections and inflammatory conditions in children."
- "Probiotic bacteria survive the gastrointestinal tract and "settle" in the gut for a while and can help in maintaining a healthier and more resistant flora. However, it is important that there is a steady supply of further bacteria, as they will disappear if more are not added."

1.3 Specific conditions of use as proposed by the notifier

One portion per day is recommended on the packet for children over 1 year, for the oatmeal porridge above 1.5 years. One portion consists of 1 dl water and 35g (5 dessert spoonfuls) of cereal powder. The number of viable cells of the culture in a portion (35g) of the cereal powder has been given as 10^8 cfu.

The shelf life of the product is 1 month after opening, provided it is kept dry and at or below room temperature.

2 Data sources

Articles and reports provided by the notifier (see Appendix III) have been assessed when the products in question and the appropriate probiotic strain have been studied, i.e. only articles on *Lactobacillus paracasei* ssp. *paracasei* F19 has been included. Human studies have been assessed when relevant for children 12 months to 3 years.

Additional literature search in MEDLINE and EMBASE using the map terms *Lactobacillus* OR probiotics and F19 OR F-19 has been conducted. From the MEDLINE and EMBASE search all relevant human studies investigating positive or negative health effects from F19 in English, Norwegian, Danish and Swedish were assessed. Articles and reports investigating

F19 in cereals and preferably in the relevant age group (1 to 3 years) have been considered most relevant. Few studies have investigated F19 in humans, and some relevant animal studies have therefore also been included.

Only articles published in peer-reviewed journal have been considered. Thus studies that are only published in the supplement Microb Ecol Health Dis 2002 Suppl 3, which is not peer-reviewed, should not be taken into consideration. However, since there are very few studies involving F19, children and safety, some of the studies in the above mentioned supplement are nevertheless commented upon.

Background papers used in this assessment are previous opinions on probiotics from EFSA related to F19, QPS and health claims.

- Opinion on the substantiation of health claims related to *Lactobacillus casei* F19 (LMG P-17806) and bowel motor function. (EFSA, 2009b)
- Opinion on the maintenance of the list of QPS (Qualified Presumption of Safety) microorganisms intentionally added to food or feed. (EFSA, 2009a)
- Opinion on the substantiation of health claims related to non-characterised microorganisms. (EFSA, 2009c)
- Draft guidance on the scientific requirements for health claims related to gut and immune function. (EFSA, 2010)

Other assessments and opinions from VKM and EFSA that have been valuable background papers in this assessment are listed in Appendix IV.

3 Hazard identification and characterisation

EFSA has recently established a draft for guidance on the scientific requirements for health claims related to gut and immune function , and decided to use FAOs criteria for characterisation of probiotics (EFSA, 2010). In the EFSA guidelines it is mentioned:

“ microorganisms (e.g. bacteria, yeast), should be sufficiently characterised (genetic typing) at strain level by internationally accepted molecular methods and strains should be named according to the International Code of Nomenclature. Strains should be deposited in an internationally recognized culture collection (with access number) for control purposes. For manufacturing processes, information should be provided to show consistency in the final product for those characteristics considered pertinent to the claimed effect. The characterisation should also be sufficient to allow control authorities to verify that the food/constituent which bears a claim is the same one that was the subject of a community authorisation.”

According to the FAO “Guidelines for the evaluation of probiotics in food”, strains should be identified at species level by DNA-DNA hybridisation or 16S rRNA sequence analysis. Strains should be characterised at strain level by DNA macro-restriction followed by Pulsed-Field Gel Electrophoresis (PFGE), Random Amplification of Polymorphic DNA (RAPD), amplified rDNA restriction analysis (ARDRA) or other internationally accepted genetic typing molecular methods (FAO, 2002).

3.1 Specific properties of F19

The FAO guidelines suggest the following definition of probiotics: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host".

3.1.1 Origin of the F19 strain

Information concerning the origin of the strain is divergent in different publications. The following origins have been published:

- Colonic mucosa in healthy subjects (Kruszewska et al., 2002).
- Deep colonic mucus layer in non-gastrointestinal diseased patients *post mortem* (Ljungh et al., 2002).
- Small intestine in a human subject (Crittenden et al., 2002).

3.1.2 Determination of the presence of plasmids, insertion sequence element, transposons, integrons and other transposable elements

According to Morelli and Campominosi, the F19 strain contains three plasmids of 2.2, 6.5 and 9.0 kb (Morelli and Campominosi, 2002). Additional information on the plasmids is given in the patent application where it is stated "and containing three plasmids having a size of 2.2, 4.36 and 9.1 kb, respectively" (*http://www.freepatentsonline.com/6599504.html*).

According to the notifier, the genome of the strain has been sequenced, but the sequence data has not been made available. As we do not have access to the sequence data we have no information regarding the presence of insertion sequence elements (IS-elements), transposons, integrons or other mobile genetic elements in the F19 strain.

3.1.3 Antimicrobial resistance properties of F19

Information concerning the antimicrobial resistance properties of F19 is divergent in 2 different publications (Morelli and Campominosi, 2002). Patent application *http://www.freepatentsonline.com/6599504.html*; Arla).

According to Morelli and Campominosi, F19 exhibits resistance to vancomycin, gentamicin, colistin, kanamycin, streptomycin, and polymixin B (Morelli and Campominosi, 2002).

According to Arla patent application (*http://www.freepatentsonline.com/6599504.html*), F19 exhibits resistance to, gentamycin, colistin, kanamycin, streptomycin, trimethoprim and cefotaxime, azteronam, ceftaxidime, cefoxitin, polymixin B and vancomycin. In the same patent application F19 is described to be susceptible to eleven antibiotics including streptomycin, penicillin G, ampicillin, bacitracin, clindamycin, chloramphenicol, erythromycin, rifampicin, tetracycline and trimethoprim.

3.1.4 Pathogenic criteria

Some probiotic strains may be able to translocate. However, a search in PubMed revealed no publication in which this question was addressed for F19, indicating that it might not have been investigated.

The possibility that probiotics may be able to aggregate platelets *in vivo* was recently commented upon (Halvorsen et al., 2009). Studies have demonstrated that certain *Lactobacillus* strains (e.g. belonging to the species *L. rhamnosus* and *L. paracasei* ssp. *paracasei*) isolated from patients with endocarditis possess pathogenic traits such as platelet aggregation, binding to fibronection and collagen, and production of enzymes enabling the breakdown of human glycoprotein and the synthesis of human fibrin clots (Harty et al., 1994).

To the best of our knowledge, there are no reports of investigations into whether F19 possess such pathogenic traits.

3.1.5 Potential pathogenicity

Lactobacilli are ubiquitous commensals of the normal human flora. Although usually considered as extremely rare causes of infections in humans, they have occasionally also been involved in serious infections. They have been associated with septicaemia and endocarditis and some cases of severe localised infections like meningitis and abscesses in lung or liver *(Husni et al., 1997).*

The species involved were mainly *L. rhamnosus* and *L. casei,* but also include some cases with *L. paracasei.* The great majority of the patients had severe underlying diseases and the role of *Lactobacillus* as a potential pathogen was linked to immunosuppression, use of broad spectrum antibiotics and surgery. The patients were mainly elderly, but some isolations were from children (Cannon et al., 2005).

An evaluation of *Lactobacillus*-induced bacteraemia in Stockholm during the period 1998-2004 identified 71 cases (< 1% of the total number of bacteraemia cases) (Sullivan and Nord, 2006). The majority of cases were caused by *L. rhamnosus* and *L. paracasei* ssp. *paracasei*, but none of the strains were found to be identical to probiotic strains.

However, a retrospective study from Finland identified 89 cases of *Lactobacillus* bacteremia between 1990 and 2000. 47 of the strains were identified to species level, 25 of whom were identified as *L. rhamnosus* (11 of these as *L. rhamnosus* GG - coinciding with a general increase in consumption of probiotics) (Salminen et al., 2004).

One article presents two cases of *Lactobacillus rhamnosus* GG sepsis directly associated with the use of probiotic therapy (Land et al., 2005). Both cases were children (6 weeks and 6 years old respectively) and both had severe underlying diseases.

Lactobacilli do not readily grow on bacteriological media typically used for clinical specimens, and may therefore be overlooked by bacteriological examinations. On the other hand, as ubiquitous commensals they may also contaminate bacteriological specimens, and the significance when isolated from infectious sites may therefore also be easily overestimated. The clinical significance of such strains isolated from normally sterile sites is therefore a subject of ongoing debate (Cannon et al., 2005).

3.1.6 Summary specific properties of F19

In summary, F19 is a well-characterised strain of *L. paracasei* ssp. *paracasei.* It contains 3 plasmids, but there is no information about the presence of other mobile genetic elements. Like many lactobacilli, the strain is resistant to several antimicrobials of clinical importance, in particular vancomycin. No information is available about possible pathogenic properties of this strain. Lactobacilli are occasionally isolated from clinical specimens, but the strain F19 has not been specifically implicated.

3.2 Adaptive properties of F19 in the gastrointestinal tract and effect on epithelial cells

3.2.1 Resistance to gastric acidity

Following ingestion and swallowing, bacteria enter the extremely acidic environment of the stomach which is normally about pH 2-3, but may be as low as 1. *In vitro* tests are mostly based on exposing the bacteria culture to a pH between 2 and 3 for varying lengths of time, and the methodology used varies. Usually acidified MRS (de Man, Rogosa and Sharpe) broth is used, but also acidified MRS agar, phosphate buffered saline or HCl solutions have been used. The exposure time varies from 1 to 10 hour but considering the short time that food is in the stomach three hours is probably the most relevant time.

There are no published dose-response studies of F19 in children, and no general opinion on doses necessary to survive the acid environment in the stomach.

According to the Arla patent application (*http://www.freepatentsonline.com/6599504.html*), the strain is characterised by being tolerant *in vitro* against HCl and gastric juice.

In vitro studies by Ljungh *et al*. showed that F19 tolerated pH 2.5 for one hour (Ljungh et al., 2002). Dunne *et al.,* 2001 also showed that F19 tolerated pH 2.5 for one hour, but were severely reduced after 5 minutes at pH 1.2, and the strain could not be isolated after 30 min (Dunne et al., 2001). The clinical relevance of these findings is unclear.

3.2.2 Bile salt resistance

Bacteria that survive the acid conditions in the stomach enter the duodenum in a stressed state and then encounter a more neutral pH and bile salts.

The physiological concentration of bile salts is around 0.3%. *In vitro* testing for bile tolerance usually employs an appropriate broth (usually MRS broth) to which bile salts have been added. Concentrations used in various studies range from 0.3 to 7.5% and survival has been assessed after periods ranging from one to 48 hours.

F19 survived 20% bile for two hours according to Ljungh *et al*. (Ljungh et al., 2002) and according to (http://www.probioticblends.com/docs/probiotic-l-paracasei-f19.pdf) this strain was able to actually grow in bile salt concentrations of 0.3 and 0.5%, and numbers remained stable at an exposure to 5% for 8 hours. Dunne *et al*. reported that several strains tested, including F19, could grow in "physiologically relevant concentrations of human bile" (Dunne et al., 2001).

According to the Arla patent application (*http://www.freepatentsonline.com/6599504.html*), the strain is characterised by being tolerant *in vitro* against HCl and gastric juice and by tolerating bile salts without deconjugating them and by having the ability to assimilate cholesterol.

During the first years of life (i.e. up to 3 years), the secreted bile salts change in their composition and conjugating properties. We are not aware of any *in vitro* study of F19 using types of bile salts relevant for small children or of any study showing assimilation of cholesterol by F19 *in vivo.*

3.2.3 Adherence to mucus and/or human epithelial cells and cell lines

The ability to adhere to intestinal surfaces is thought to be important for the efficacy of probiotic strains. The mucus covering the epithelial cells is the initial surface that ingested microorganisms confront in the human gut, and is considered an important site for bacterial adhesion. It is believed that mucus has receptors mimicking the epithelial cells, to which the bacteria adhere. Mucus is, however, continually subjected to degradation and washed away with the luminal contents (including their content of microorganisms). In this way bacteria may consequently be prevented from reaching the enterocytes.

Assessment of bacterial adhesion *in vivo* is difficult, and *in vitro* models are therefore commonly applied for this purpose.

The ability of different probiotic strains, including F19 to adhere to mucus isolated from infants (newborn, 2 and 6 months old) and adults was examined *in vitro*. F19 was shown to adhere rather weakly and significantly weaker to mucus from infants than from adults (appr. 6% versus 9.7%) (Kirjavainen et al., 1998).

Another *in vitro* study was designed to assess whether the adhesion to mucus was affected by an ongoing rotavirus infection (Juntunen et al., 2001). The rotavirus infection did not decrease the production of fecal mucin and the adherence of F19 was not affected. The overall results were similarly to those found by Kirjavainen.

The fact that probiotic bacteria in general have a transient pattern of presence in feces, may indicate that adherence may not be of critical importance for their efficacy. The survival and ecology of F19 in human subjects have been investigated in a multicentre European research project, PROBDEMO, and it was shown that in doses between 10^8-10^{10} cfu, F19 only transiently colonised the colonic lumen and the mucosa (PROBDEMO, 2002). In some young children the colonisation lasted for up to 2 weeks after cessation of intake and 2 elderly subjects were still colonised after 8 weeks.

In another study F19 remained in 8% and 20% in children and elderly persons respectively for several weeks (Sullivan *et al.*, 2002). In these cases F19 seems to have been established as part of the normal microbiota.

We are unaware of any *in vivo* studies concerning the specific ability of F19 to adhere directly to human enterocytes or mucus.

3.2.4 Physiological aspects

Commensals, including probiotics have been found to "cross-talk" with enterocytes. The results from comparative studies in conventional and germfree mice demonstrated that F19 influenced the expression of "a number of genes involved in essential physiological functions such as immune response, regulation of energy homeostasis and host defense" (Nerstedt et al., 2007). However, this response was different in the germfree vs. conventional mice. The authors underlined that "any impact of *Lactobacillus* feeding is likely to be affected by factors such as age, sex, health status, already existing gut microflora etc".

3.2.5 Summary adaptive properties of F19 in the gastrointestinal tract and effect on epithelial cells

According to the Arla patent application (*http://www.freepatentsonline.com/6599504.html*), the strain is characterised by being tolerant *in vitro* against HCl and gastric juice and by

tolerating bile salts without deconjugating them and by having the ability to assimilate cholesterol. However, during the first years of life (i.e. up to 3 years), the secreted bile salts change in their composition and conjugating properties. We are not aware of any *in vitro* study of F19 using types of bile salts relevant for small children.

The ability to adhere to intestinal surfaces is thought to be important for the efficacy of probiotic strains. The survival and ecology of F19 in human subjects have been investigated in a multicentre European research project, PROBDEMO, and it was shown that F19 only transiently colonised the colonic lumen and the mucosa. Another *in vitro*-study showed that F19 adhered rather weakly to mucus and significantly weaker to mucus from infants than from adults (Kirjavainen et al., 1998).

Commensals, including probiotics have been found to "cross-talk" with enterocytes. In animal studies, F19 was shown to influence the expression of "a number of genes involved in essential physiological functions such as immune response, regulation of energy homeostasis and host defense". Any impact of *Lactobacillus* feeding is likely to be "affected by factors such as age, sex, health status, already existing gut microflora etc" (Nerstedt et al., 2007).

3.3 Health effects of intake of F19

Positive and adverse health effects of probiotics are considered to be mainly species and strain specific and therefore only studies investigating the specific strain are relevant in assessing benefits and risks from the products in question. Moreover, the food matrix may be of importance when assessing safety and efficacy, although this is probably not as significant as strain specificity.

Articles and reports investigating F19, preferably in the relevant age group (12 months to 3 years) have been considered sufficiently relevant to be included in this report.

West *et al*. studied F19 in cereals for children 4 to 13 months (West et al., 2008), which make these studies relevant for the product in question although the present product is intended for use in children 12 months and older. This is the only report available studying the processed cereal-based food containing F19, but the product was only given to children in the relevant age group.

Table 2: Studies with F19 given to children below 3 years of age.

Author	Journal	Number of children	Age
Sullivan, *et al*.	Micr J Health and Dis, 2002 Suppl 3	77 (16 drop outs) 30 active 31 placebo	12 months (SD1.4) 13 months (SD2)
Crittenden *et al.*	Micr J Health and Dis, 2002 Suppl 3	61	12 – 18 months
West, Christina	Umeå University medical dissertations, 2008	179 (8 drop outs) 89 active 90 placebo	4-13 months
Kirjavainen *et al.*	Microbiology letters, 2006	56	0 – 6 months
Juntunen, *et al.*	Clinical & Diagnostic Laboratory Immunology 2001	30 20 (rotavirus) + 10 (control)	6 – 42 months

3.3.1 Safety studies

Grzeskowiak *et al*., suggest that different sources of the same probiotic may have significantly altered strain properties and underlined the importance of control of the manufacturing process and the food matrix (Grzeskowiak et al., 2011). The notifier has not supplied any safety studies of F19 in processed cereal-based products.

The EFTA PROSAFE workshop (Product Safety Enforcement Forum of Europe) discussed recommendations on taxonomy, antibiotic resistance, *in vitro* assessment of safety and recommendations for *in vivo* assessment of safety of probiotics used for human consumption (PROSAFE, 2008). Consensus was reached for approving the necessity of bacterial colonisation studies in humans in a randomised placebo controlled design.

The PROBDEMO project was designed to also unravel safety issues, and the results from project were published in supplement 3 Microb Ecol Health Dis. 2002. No adverse effects were noted after feeding young children with freeze dried F19 in capsules (Sullivan and Nord, 2006). In the same project Sullivan *et al*. investigated the impact on the intestinal microflora by feeding F19 to small children (12-18 months).

As part of a multicentre European project F19 was included in four pilot feeding studies. The target groups in these studies ranged from infants to elderly and included both healthy subjects and individuals with mild health disorder (milk hypersensitivity and *H. pylori* infection). No adverse effects attributable to the administration of F19 were observed in any of the pilot studies (Crittenden et al., 2002).

There are no studies designed to unravel the long-term safety aspects of feeding F19 to young children on a daily basis. However, the studies by Christina West reported in her doctoral thesis describe children fed processed cereal-based baby food containing F19 from weaning at 4 months until 13 months of age (West et al., 2008). The supplemented processed cereal-based baby food was well tolerated.

F19 has been given to mice, both ordinary and mice treated with cyclophosphamid (a substance making the mice sensitive to infections). In spite of this treatment, the mice showed no side effects attributable to the intake of F19 (Wang et al., 1998).

Nerstedt *et al.* showed that the genes in the intestinal cells were switched on/off in laboratory mice exposed to F19 (Nerstedt et al., 2007). The mere fact that F19 originate from human intestinal microbiota makes it reasonable to assume that similar microbe/cell interactions may take place in children. However, we are not aware of studies, *in vitro* or *in vivo,* in which such interactions have been studied on human cells.

3.3.1.1 *Summary safety studies*

Few studies are designed to unravel safety issues concerning feeding F19, and there are no studies on long-time safety. Furthermore, the only studies on short-time safety have been published in a non peer-reviewed supplement to an otherwise peer-reviewed journal (Supplement 3 Microb Ecol Health Dis. 2002). Studies focusing on safety do not demonstrate any short term adverse effects.

3.3.2 Studies on beneficial effects

3.3.2.1 *Effect on development of allergies and atopic eczema*

A beneficial effect with respect to the development of atopic eczema following the feeding of F19 to infants from weaning at 4 months to 13 months of age was demonstrated by West *et al.* (West et al., 2008). Healthy infants (n=179) delivered at term were included in the study, 8 drop outs due to removal of consent. Eighty-four infants were given F19 in processed cereal-based baby food from 4 months of age and 87 infants constituted the placebo group. The incidence of eczema was 11% and 22% in the treatment and placebo group respectively (p=0.049). However, a beneficial effect on development of allergies and atopic eczema from processed cereal-based baby food fortified with F19 from 12 months onwards has not been investigated. Furthermore, the demonstrated effect was only marginally significant and the clinical significance of these results is therefore questionable.

There was no indication that allergic sensitisation was reduced in the probiotic group.

3.3.2.2 *Effect on infections*

West *et al*. could not demonstrate any difference in the number of days of infection between the children fed processed cereal-based baby food fortified with F19 and the placebo group (West et al., 2008). However, the probiotic group did have marginally significantly fewer

days with the administration of antibiotics compared with the placebo group (p=0.044). This finding may be questioned since most infections in young children are viral and thus not to be susceptible to antibiotics.

3.3.2.3 *Summary of studies on beneficial effects*

F19 has, in one study, been shown to result in less atopic eczema when given to infants from weaning at 4 months until 13 months of age (West et al., 2008). Based on a marginal statistical significance in one study no conclusions should be drawn. The group of children given the fortified processed cereal-based baby food was less prone to be given antibiotics than the placebo group, but no significant positive effect on infections was demonstrated.

There is no documentation for effect on general maintenance of a healthier and more robust gut flora, nor documentation of reduced allergic sensitisation.

4 Exposure assessment

Intake of processed cereal-based foods in children above 12 months in Norway (Spedkost - 12 months and Småkost - 2 years)

The notifier has informed that a portion of cereal contains 10^8 cfu of viable cells of F19. According to the wording on the package, one portion of this cereal per day is recommended. One portion of the prepared cereal weighs about 135 g.

The labelling contains no warning about serving more than one portion per day.

Data on intake of processed cereal-based food in 1 year olds and 2 year olds are available in Spedkost and Småkost (Helsedirektoratet, 2009). At the age of 12 months 87% of the infants were given porridge on a daily or weekly basis. Most infants (82%) were given commercially manufactured processed cereal-based baby food. Among these, average intake (ready to eat) was 265 g/day. An intake of 265g/day would therefore be equivalent to approximately 2 x 10^8 cfu in the notified products.

As described above more than 80% of 12 month old children were fed processed cereal-based baby food and the daily intake was relatively high. Thus a processed cereal-based baby food containing probiotic bacteria could provide a daily intake containing a high bacterial load, and thus disturbing the evolution of a normal balanced bacterial diversity. There are no published dose-response studies of F19 in children.

5 Risk and benefit characterisation

Information concerning the origin of the strain is divergent in different publications, as mentioned in section 3.1.1. The source of the strain had been given as the colonic mucosa in healthy subjects (Kruszewska et al., 2002), the deep colonic mucus layer in non-gastrointestinal diseased patients *post mortem* (Ljungh et al., 2002) or from the small intestine in a human subject. (Crittenden et al., 2002). VKM finds this divergence disconcerting since it indicates a lack of dependable traceability for the strain. Also it is not possible to isolate one strain from several subjects.

The information from the notifier regarding the genetic stability of F19 in the two products during processing and storage is considered as insufficient and does not allow any conclusions to be drawn.

5.1 Safety

The four studies included in the doctoral thesis of Christina West *et al*. are the only studies in humans pertaining to benefit and safety of F19 in small children (West et al., 2008). Two of these articles are to date published in peer reviewed journals. The investigations were not designed to unravel safety issues, but no short term adverse effects were observed. Long term adverse effects were not studied as the intervention ended at 13 months of age. There are no studies investigating the effect of F19 on infections in the relevant age group.

A daily supply of a monoculture of a particular bacterial strain in large quantities to an age group without a fully established intestinal flora may have unknown adverse effects. There is however, to our knowledge, no studies investigating possible short or long term adverse health effects of F19 in processed cereal-based baby food given to children 13 months onwards.

In the PROBDEMO project it was concluded that in order to be able to draw any conclusions regarding the safety of F19, there is a need for randomised placebo-controlled studies in larger populations (PROBDEMO, 2002). Differences in establishment of a normal gut flora should be investigated. Long term follow up including clinical parameters is missing.

5.2 Possible association between antimicrobial resistance phenotype and resistance genes

Information provided by the notifier regarding possible association between antimicrobial resistance phenotype and resistance genes is insufficient and partly inconsistent. The documentation obtained is not conclusive regarding the antibiotic resistance pattern of the strain used in the products in question , as the information on different antibiotics also are partly inconsistent. The information about specific localisation (chromosomal, plasmid) of the resistance genes is not sufficient. Furthermore, there is lack of data regarding possible association between the resistance genes with IS-elements, transposons, integrons, and other mobile genetic elements in F19.

Morelli *et al*. concluded that all plasmid-cured derivates (cured for 6.5 and 9.0 kb plamids) of F19 exhibited the same resistance profile as non plasmid-cured F19 (Morelli and Campominosi, 2002). The authors concluded that this is an indication that the resistance phenotype is not encoded by plasmids with molecular weights 6.5 and 9.0 kb. This conclusion may be correct, although it does not exclude that genes encoding resistance to these antimicrobial agents are located on other transposable genetic elements (including the smallest plasmid). This has not been investigated, either by Morelli *et al*. (Morelli and Campominosi, 2002), or by others. Molecular methods such as Polymerase Chain Reaction (PCR) may confirm the presence of resistance genes in F19. Should this be the case, the localisation of the gene(s) on plasmid(s) or chromosome should be confirmed by hybridisation study.

The notifier has been given the opportunity to clarify this inconsistency and to supply additional documentation on several occasions.

According to the guidelines for probiotics in food (FAO, 2002), it is recommended that use of probiotic bacteria should be restricted to those strains that do not harbour transmissible drug resistance genes encoding resistance to clinically used drugs.

5.3 Interference with host genes

In the first 2-3 years of a child's life, a considerable number of bacterial species (possibly more than 2000) become established in the gastrointestinal tract, and each of them may "crosstalk" with the enterocytes, thereby shaping the phenotypic expression of the host. The data presented by Nerstedt demonstrate that F19 is able to "crosstalk" with enterocytes in mice and that the result of the "crosstalk" depends upon the microbiota present (Nerstedt et al., 2007). Whether F19 has a similar "crosstalk-profile" in humans is unknown. However, as the strain is originally of human origin, it seems reasonable to assume that such "crosstalk" may take place. Thus, before giving F19 on a daily base for months and years, it seems reasonable to ask for additional molecular and physiological studies to unravel the functional impact of possible changes in genetic expression of the host.

5.4 Pathogenicity

EFSA has recommended the Qualified Presumption of Safety approach in order to assess the safety of microbial species (not strains) used in food, and *Lactobacillus paracasei* has achieved the status QPS at species level (EFSA, 2009a). VKM's Panel on Biological Hazard has however commented upon EFSAs QPS-opinion:

"The decision of whether or not to award QPS status to microorganisms should remain with risk assessors, and should be subject to review at pre-determined intervals, as well as triggered by specific events, for example if a specific alteration occurs, including acquisition of new, relevant knowledge. Furthermore, consideration should be given to whether QPS should be applied to microorganisms with respect to particular consumer groups (or whether particular, potentially-vulnerable consumer groups should be excluded)" (VKM, 2007).

5.5 Infections

Infections caused by *Lactobacillus* are considered extremely uncommon among immunocompetent people. The wide distribution of *Lactobacillus* and the relatively few infections they cause indicates that these bacteria have very low virulence in healthy humans. This lack of pathogenicity seems to extend across all age groups. EFSA has stressed that human infections should remain a topic for surveillance (EFSA, 2009c).

Lactobacillus infections do occasionally occur, mainly as bacteremia, endocarditis and localized infections (e.g. abscesses, peritonitis, meningitis) in patients with severe underlying diseases. Most of them are elderly, but children are not excluded. The species most often isolated are *L. casei* and *L. rhamnosus*, followed by *L. paracasei*.

The increasing use of immunosuppressive therapy and broad spectrum antibiotics ineffective against *Lactobacillus* might increase the importance of these bacteria as possible pathogens.

5.6 Benefit

In order to be able to draw any conclusions regarding beneficial effects of F19, there is a need for randomised placebo-controlled studies in larger populations in the relevant age group.

5.7 Health claims

According to EFSA, *Lactobacillus paracasei* ssp. *paracasei* F19 is sufficiently characterized (EFSA, 2009c). The documentation provided is however not sufficient to claim positive health effects and thus F19 is not proven to be probiotic.

No documentation is provided which may substantiate the claims on the package or website for the relevant products that processed cereal-based baby food fortified with F19 "keeps the gut in balance" or "contributes to maintain a healthier and more robust gut flora".

It is not the mandate of this report to evaluate the health claims related to the products as these health claims are assessed by EFSA. The claimed health effects of the product in question are similar to those assessed by EFSA for F19 and other probiotic bacteria. None of the health claims pertaining to any strain of probiotic bacteria have so far been accepted by EFSA. In a scientific opinion related to F19 and bowel movement function, it was concluded that a cause and effect relationship between the consumption of F19 and improvement of bowel movement function was not established.

Incorrect health claims may give the consumers false expectations, and in worst case promote excessive use of a product that is not sufficiently documented or proven safe.

5.8 Dose-response studies

There are no dose-response studies on F19. The response to a probiotic strain depends on both the survival of the probiotic through the stomach and duodenum and on the actual efficacy of the strain concerned in the intestine. There is no consensus of opinion as to the dose of probiotics needed in order to achieve an effect, but some studies suggest 10^8 cfu viable cells per day for adults. The bacterial dose contained in one portion of processed cereal-based food is 10^8 cfu cells, which could arguably be a high dose for small children. Therefore, the number of viable cells added to the notified products is presumably not dictated by a documented need for a specified dose of viable bacteria in order for a potential health effect to be observed in small children.

5.9 Contraindications for use, special groups

The notifier has applied for 2 cereal-based products containing F19. As the clinical documentation is restricted to one group of healthy children receiving one of the products up to 13 months of age, there is no data related to F19 mentioning specific groups of children in which F19 should be avoided.

However, based on experience with other probiotics, it is reasonable to underline that F19 should not be given to immunocompromised or seriously ill children (VKM, 2009).

6 Data gaps

- Dose – response studies on children
- Studies on genetic stability
- Studies on adherence to enterocytes and/or mucus "colonisation"
- Studies focusing on the impact of different deliverance matrixes
- Double-blind placebo-controlled clinical studies focusing on safety, short- and long term.
- Double-blind placebo-controlled studies on positive health effects in children 1 – 3 years of age
- F19, when given as processed cereal-based food containing *Lactobacillus paracasei* ssp. *paracasei* F19, may switch on/off host genes of physiological importance, and the need for further studies are too obvious to be ignored.
- Correct antimicrobial resistance profile
- Information regarding type of antimicrobial resistance properties (inherent or acquired)
- Localisation of antimicrobial resistance genes (plasmid or chromosome or both)
- Presence of mobile genetic elements like Insertion elements (IS), integrons, transposons, gene cassettes, etc
- Before giving *Lactobacillus paracasei* ssp. *paracasei* F19 on a daily base for months and years, it seems reasonable to ask for additional molecular and physiological studies to unravel the functional impact of possible changes in genetic expression of the host.
- Differences in establishment of a normal gut flora should be investigated. Long term follow up including clinical parameters is missing.
- Molecular and physiological studies to unravel the functional impact of possible changes in genetic expression of the host

7 Answer to the terms of reference

1. What benefit can children (1 to 3 years) have from consuming processed cereal-based food containing *Lactobacillus paracasei* ssp. *paracasei* F19?

There is only one study indicating a positive effect of *Lactobacillus paracasei* ssp. *paracasei* F19 given to infants 4 – 13 months on the development of eczema.

The positive health effects claimed for processed cereal-based food containing *Lactobacillus paracasei* ssp. *paracasei* F19 are not scientifically substantiated. Thus the claims "good for gut health", "strengthens the immune system" or "has a health promoting effect for children and adults" are not evidence-based. No conclusions can be drawn as to positive effects from F19 in children 1 – 3 years of age.

2. What impact can the addition of *Lactobacillus paracasei* ssp. *paracasei* F19 to processed cereal-based food for children (1 to 3 years) have on the development of allergy?

There is no documentation of any effect on the development of allergic sensitization in children at 13 months of age, (West et al., 2009) and no other documentation on allergic sensitization in children 1 – 3 years having been fed *Lactobacillus paracasei* ssp. *paracasei* F19 have been provided.

3. Are there any contraindications regarding the consumption of processed cereal-based food containing *Lactobacillus paracasei* ssp. *paracasei* F19 for children (1 to 3 years)?

There is no answer to this question due to absence of long term studies in children receiving F19 on a daily base.

4. Is there any risk that the consumption of processed cereal-based food containing *Lactobacillus paracasei* ssp. *paracasei* F19 can lead to a spreading of antimicrobial resistance to other members of the gut microbiota?

This question cannot be answered since information provided by the notifier regarding possible association between antimicrobial resistance phenotype and resistance genes is insufficient and partly inconsistent.

In general and in the case of the presence of resistance gene(s) in F19, the risk of transfer of such gene(s) to the resident microbiota and pathogenic bacteria and increased development of antimicrobial resistance in bacteria can not be excluded.

5. What possible negative health effects are correlated to the daily consumption of processed cereal-based food containing *Lactobacillus paracasei* ssp. *paracasei* F19? Furthermore, will an increased consumption (amount and frequency) result in pronounced effects?

There are no published dose-response studies of F19 in children, neither regarding survival of F19 in the gastrointestinal tract, nor possible negative health effects. Thus the potential for negative health effects as e.g. spreading of antimicrobial resistance or unfavorable impact on the genetic expression by the host related to the frequency and/or dose of a monoculture of F19 cannot be assessed.

6. Do the products contain ingredients that can have prebiotic effect?

Any food products which contain whole grains contain fibre components, some of which may not be hydrolyzed by human digestive enzymes. They will therefore pass undigested into the colon where they may be metabolised. Such components may stimulate beneficial members of the colon flora and could then be considered to have a prebiotic effect. To the best of our knowledge, F19 has not been assessed for its ability to metabolise such components.

Appendix I. Norwegian terms of reference:

1. Hvilken nytte kan barn fra 1 inntil 3 år ha av å bruke barnegrøt tilsatt *Lactobacillus paracasei* ssp. *paracasei* F19?

2. Hva betyr tilsetning av *Lactobacillus paracasei* ssp. *paracasei* F19 i barnegrøt for barn fra 1 inntil 3 år for utvikling av allergi?

3. Er det noen kontraindikasjoner ved bruk av barnegrøt som inneholder *Lactobacillus paracasei* ssp. *paracasei* F19 for barn fra 1 inntil 3 år?

4. Er det risiko for at bruken av *Lactobacillus paracasei* ssp. *paracasei* F19 i barnegrøt kan føre til spredning[3] av antimikrobiell resistens?

5. Hvilke eventuelle negative effekter vil bruk av *Lactobacillus paracasei* ssp. *paracasei* F19 (mengde og frekvens) i barnegrøt for barn fra 1 inntil 3 år kunne ha?

6. Inneholder produktene ingredienser som kan virke som prebiotika?

[3] Overføring av resistensgener til andre tarmbakteriearter

Appendix II. Påstander i forslag til merking av produktene på norsk samt tekst fra virksomhetens hjemmeside

På pakningene:

Flerkornsgrøt med probiotika (For barn fra 12 måneder)

Sunn mat for små mager.

Probiotika holder magen i balanse.

Flerkornsgrøt med probiotika er tilsatt melkesyrebakterier som naturlig finnes i tarmen vår. Et ekstra tilskudd av disse bidrar til å holde magen i balanse, og bedre immunsystemet.

Havregrøt med probiotika (For barn fra 1,5 år)

Havregrøt med probiotika er tilsatt melkesyrebakterier som naturlig finnes i tarmen vår. Et ekstra tilskudd av disse bidrar til å holde magen i balanse, og bedre immunsystemet.

På hjemmesiden:

Havregrøt med banan og bringebær inneholder probiotiske melkesyrebakterier som har vitenskapelige dokumenterte egenskaper. Probiotiske melkesyrebakterier finnes naturlig i tarmen vår, og et ekstra tilskudd av disse kan bidra til å opprettholde en sunnere og mer motstandsdyktig flora. Mage- og tarmsystemet er kroppens viktigste immunsystem og probiotika holder magen i balanse.

I barnehagen, og spesielt den første tiden, er det mange barn som blir syke med forkjølelse eller får mageproblemer.

For å hjelpe immunforsvaret til de små, kan det være lurt å gi barna et ekstra tilskudd av probiotiske melkesyrebakterier. Probiotika har vist seg å ha en helsefremmende effekt på barn og voksne.

Mange barn har hatt sitt aller første møte med barnehagen denne høsten. Barnehagestart er en viktig og stor hendelse for mange barn. Det er deres første møte med et selvstendig liv uten foreldrene til å beskytte seg. Dette er en tid da en skal lære seg å dele leker og ha tålmodighet ved matbordet der det er flere mager å mette enn armer til å servere.

Den første tiden i barnehagen er også møte med mange nye bakterier, samtidig som høstens kalde og fuktige vær setter immunforsvaret på prøve.

Helt fra spedbarnet forlater det sterile miljøet i livmoren og møter en verden full av bakterier, starter etableringen av komplekse bakteriekulturer på hud og slimhinner, som munn, svelg og tarmkanalen. Dette kalles "normalflora".

Bare i tykktarmen kommer mer enn 500 ulike bakteriearter til å etablere seg. Denne prosessen skjer suksessivt og en "voksen" tarmflora oppnås ikke før i 3-4 års alderen. Bakteriene i tarmfloraen har stor betydning for stimuleringen av vårt immunsystem. De fleste av tarmens bakterier er "snille" og gir sjelden eller aldri opphav til infeksjoner.

En sammensatt tarmflora gir beskyttelse mot kolonisering av farlige bakterier, gjennom å skape konkurranse om næringen og plassen. Tarmbakterienes evne til å stimulere immunsystemet og konkurrere med farlige bakterier har ført til utviklingen av probiotika.

Probiotiske melkesyrebakterier er ufarlige bakterier som kan tilsettes mat og drikke. Forskning viser at en del probiotika har god effekt på visse infeksjoner og inflammatoriske tilstander hos barn.

Probiotiske bakterier overlever mage- tarmkanalen og "bosetter" seg i tarmen i en periode og kan bidra til å opprettholde en sunnere og mer motstandsdyktig flora. Men det er viktig med stadig tiførsel, da bakteriene forsvinner hvis det ikke tilføres flere.

Appendix III. List of articles provided by the notifier

Alander, M., De Smet, I., Nollet, L., Verstraete, W., von Wright, A., Mattila-Sandholm, T. 1999. The effect of probiotic strains on the microbiota of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME). Int J Food Microbiol 46:71-79.

Björneholm, S., Eklöw, A., Saarela, M., Mättö, J. 2002. Enumeration and identification of *Lactobacillus paracasei* subsp. *paracasei* F19. Microbial Ecology in Health and Disease Suppl. (*Lactobacillus* F19 – Closing the broken circle) 3:7-13

Charteris, W., Kelly, P., Morelli, L., Collins, K. 1998a. Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. J Food Prot 61:1636-1643.

Charteris, W., Kelly, P., Morelli, L., Collins, K. 1998b. Development and application of an *in vitro* methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper gastrointestinal tract. J Appl Microbiol 84: 759-768.

Charteris, W., Kelly, P., Morelli, L., Collins, K. 2001. Quality control *Lactobacillus* strains for use with the API 50CH and API ZYM systems at 37C. J. Basic Microbiol. 41(5):241-251.

Crittenden, R., Saarela, M., Mättö, J., Ouwehand, A.C., Salminen, S., Pelto, L., Vaughan, E.E., de Vos, W.M., von Wright, A., Fondén, R., Mattila-Sandholm, T. 2002. *Lactobacillus paracasei* subsp. *paracasei* F19: survival, ecology and safety in the human intestinal tract - a survey of feeding studies within the PROBDEMO project. Microbial Ecology in Health and Disease Suppl. (*Lactobacillus* F19 – Closing the broken circle) 3:22-26.

Delia, A., Morgante, G., Rago, G., Musacchio, M.C., Petraglia, F., and De Leo, V. 2006. Effectiveness of oral administration of *Lactobacillus paracasei* subsp. *paracasei* F19 in association with vaginal suppositories of *Lactobacillus acidophilus* in the treatment of vaginosis and in the prevention of recurrent vaginitis. Minerva Ginecol. 58(3):227-231.

Fondén, R., Björneholm, S., Ohlson, K. 2002. Lactobacillus F19 - safety considerations in practice. Proceedings of the IDF Seminar on Aroma and Texture of Fermented Milk held in Kolding, Denmark, June 2002; pp 159-167; ISBN: 92-9098-036-2; Publisher: International Dairy Federation; Brussels; Belgium; 32 ref.

Fondén, R., Ohlson, K., Svensson, U. 2003. Survival instinct. Dairy Ind Int 68:17-18.

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Appendix IV. Other assessments and opinions on probiotics from VKM and EFSA.

Assessment of benefits and risks of probiotics in processed cereal-based babyfoods. *Bifidobacterium lactis* Bb12 (VKM, 2010).

The use of probiotics for patients in hospitals. A benefit and risk assessment (Halvorsen et al., 2009).

Scientific substantiation of a health claim related to LGG® MAX and reduction of gastro-intestinal discomfort (EFSA, 2008).

Scientific substantiation of a health claim related to LACTORAL (a combination of three probiotic strains: *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*) and normal functioning of the alimentary tract (EFSA, 2008).

Scientific substantiation of a health claim related to regulat®.pro.kid BRAIN and mental and cognitive developments of children (EFSA, 2008).

Scientific substantiation of a health claim related to regulat®.pro.kid IMMUN and immune system of children during growth (EFSA, 2008).

Risk assessment on use of *Lactobacillus rhamnosus* (LGG) as an ingredient in infant formula and baby foods (II) (VKM 2007).

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